10 DEC 2001

FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
PU-9925	To be assigned 10/018042

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/SE00/01174	June 21, 2000	June 30, 1999

TITLE OF INVENTION

Combinatorial Library with Particle Classes that can be Distinguished by Two Features e.g. Size, Density, Color, etc.

APPLICANT(S) FOR DO/EO/US

Nils Norrman

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

copy of this transmittal letter for charging purposes
return postcard

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/018042 To be assigned	PCT/SE00/01174	PU-9925

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☒	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$1,040.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	16 - 20 =	0	x $18.00	**$0.00**
Independent claims	2 - 3 =	0	x $84.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**
	TOTAL OF ABOVE CALCULATIONS =			**$1,040.00**
☐	Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.			**$0.00**
	SUBTOTAL =			**$1,040.00**
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +			**$0.00**
	TOTAL NATIONAL FEE =			**$1,040.00**
☐	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			**$0.00**
	TOTAL FEES ENCLOSED =			**$1,040.00**
			Amount to be: refunded	$
			charged	$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **500-588** in the amount of **$1,040.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **500-588** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Amersham Biosciences
800 Centennial Avenue
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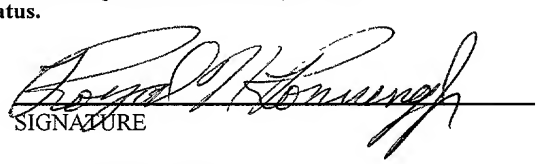
(732) 457-8423



SIGNATURE

Royal N. Ronning, Jr.

NAME

32,529

REGISTRATION NUMBER

December 10, 2001

DATE

PU-9925 #3/a

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	N. Norrman	Group Art Unit:	To be assigned
Serial Number:	To be assigned	Examiner:	To be assigned
Filing Date:	December 7, 2001		
Title:	Combinatorial Library with Particle Classes that can be Distinguished by Two Features e.g. Size, Density, Color, etc.		

FIRST PRELIMINARY AMENDMENT

Honorable Assistant Commissioner of Patents
Box Patent Application
Washington, D.C. 20231

Sir:

Please consider the following amendments and remarks in connection with the prosecution of the captioned application, which is a filing under 35 U.S.C. § 371 and claims priority to international application number PCT/SE00/01174 filed June 21, 2000. This application also claims priority to patent application number 9902479-6 filed in Sweden on June 30, 1999.

In the Claims

Please amend claim 1 as follows:

1. (once amended) A method of identifying one or more substances having affinity for a given target substance, comprising:

(i) providing a set of particle classes, each said particle class being distinguishable from [another class]other classes within the set by at least one physical property, each particle class being comprised of at least two [subclasses]sub-classes, wherein each [subclass]sub-class is distinguishable from [another subclass]the other sub-classes of said particle class by [another]a physical property, which is different from the property which distinguishes the particle class from other classes within the set, and further wherein [different from the property of said particle classes,]each [subclass comprising]sub-class contains particles having at least one of said one or more substances attached to the surface thereof as a ligand, said ligands being different from ligands attached to particles of other particle classes or [subclasses]sub-classes;

(ii) combining a plurality of classes or sub-classes to form at least one mixture,

(iii) distributing [the mixtures]said at least one mixture in separate vessels;

(iv) exposing [each]said mixture in said separate vessels to said target substance;

(v) [washing away]removing all target substance not having bound to any ligand; and

(iv) identifying to which particle [sub-class(es)]classes or sub-classes said target substance actually has (have) bound, by[the following steps:]

identifying in which vessel or vessels target substance has bound to particles in the mixture present in said vessel or vessels, identifying to which particle class said target substance has bound; and

identifying to which particle class or sub-class said target substance has bound.

Please amend claim 2 as follows:

2. (once amended) The method [as claimed in]of claim 1, wherein each particle class is characterized by one of the physical properties [in]selected from the group consisting of size, density, color and shape.

Please amend claim 3 as follows:

3. (once amended) The method [as claimed in]of claim 2, wherein each particle sub-class is characterized by one of the physical properties [in the group]selected from the group consisting of size, density, color and shape, but is difference from the property characterizing the particle class.

Please amend claim 4 as follows:

4. (once amended) The method [as claimed in]of claim 1, wherein the mixtures are formed by mixing two particle classes or sub-classes in a ratio such that the difference in [number of one class with respect to the other is detectable in the identifying step]the relative amount of each class or sub-class can be used to determine the particle class to which the target substance is bound.

Please amend claim 5 as follows:

5. (once amended) The method [as claimed in any preceding claim]of claim 1, wherein the target substance is marked so as to be detectable.

Please amend claim 6 as follows:

6. (once amended) The method [as claimed in]of claim 5, wherein the [marking is performed]target substance is marked by attaching a moiety selected from the group consisting of a fluorescent moiety, a radioactive moiety, a colored moiety.

Please amend claim 7 as follows:

7. (once amended) The method [as claimed in]of claim 5, wherein the target substance reacts with the ligand to which it binds to provide a detectable effect, such as fluorescence or color.

Please amend claim 8 as follows:

8. (once amended) The method [as claimed in any preceding claim]of claim 1, wherein the identification is performed by ocular inspection under microscope.

Please amend claim 9 as follows:

9. (once amended) The method [as claimed any preceding claim]of claim 1, wherein the identification is performed by image analysis with a computer.

Please amend claim10 as follows:

10. (once amended) The method [as claimed in any preceding claim]of claim 1, wherein said mixture is exposed to at least two target substances.

Please amend claim 11 as follows:

11. (once amended) A library of different ligands, comprising particles belonging to a plurality of particle classes, each particle class being distinguishable from [another class]the other particle classes by at least one physically distinguishable property, each particle class being comprised of at least two [subclasses]sub-classes, wherein each [subclass]sub-class is distinguishable from [another subclass]the other sub-classes by [another]a physical property of the particle class from the property which distinguishes the particle class from other classes within the set, and further wherein[, different from the property(ies) of said particle classes,] those particles belonging to [one and]the same sub-class having at lease one type of ligand attached to their surface.

Please amend claim 12 as follows:

12. (once amended) The ligand library [as claimed in]of claim 11, wherein one of said properties of the particle classes or sub-classes is the size[, suitably the diameter] of the particles.

Please amend claim 13 as follows:

13. (once amended) The ligand library [as claimed in]of claim 11, wherein one of said properties of the particle classes or sub-classes is the density of the particle.

Please amend claim 14 as follows:

14. (once amended) The ligand library [as claimed in]of claim 11, wherein one of said properties of the particle classes or sub-classes is the shape of the particle.

Please amend claim 15 as follows:

15. (once amended) The ligand library [as claimed in]of claim 11, wherein one of said properties of the particle classes or sub-classes is the color of the particle.

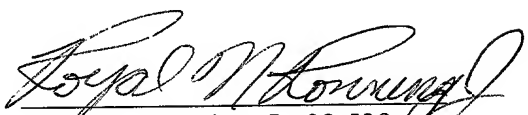
Remarks

Claims 1-16 are pending in the instant application. Applicants have amended claims 1-15 to more fully conform with U.S. practice and to delete multiple dependencies. A version of the claims marked up to show the amendments, as well as a clean version of the claims encompassing the amendments, is attached hereto.

Applicants respectfully assert that all amendments are fairly based on the specification, and respectfully request their entry.

Applicants believe that the claims, as amended, are in allowable form, and earnestly solicit the allowance of claims 1-16.

Respectfully submitted,



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Claims (marked-up version showing amendment(s))

1. (once amended) A method of identifying one or more substances having affinity for a given target substance, comprising:

(i) providing a set of particle classes, each said particle class being distinguishable from [another class]other classes within the set by at least one physical property, each particle class being comprised of at least two [subclasses]sub-classes, wherein each [subclass]sub-class is distinguishable from [another subclass]the other sub-classes of said particle class by [another]a physical property, which is different from the property which distinguishes the particle class from other classes within the set, and further wherein [different from the property of said particle classes,]each [subclass comprising]sub-class contains particles having at least one of said one or more substances attached to the surface thereof as a ligand, said ligands being different from ligands attached to particles of other particle classes or [subclasses]sub-classes;

(ii) combining a plurality of classes or sub-classes to form at least one mixture,

(iii) distributing [the mixtures]said at least one mixture in separate vessels;

(iv) exposing [each]said mixture in said separate vessels to said target substance;

(v) [washing away]removing all target substance not having bound to any ligand; and

(iv) identifying to which particle [sub-class(es)]classes or sub-classes said target substance actually has (have) bound, by[the following steps:]

identifying in which vessel or vessels target substance has bound to particles

in the mixture present in said vessel or vessels, identifying to which particle class said target substance has bound; and

identifying to which particle class or sub-class said target substance has bound.

2. (once amended) The method [as claimed in]of claim 1, wherein each particle class is characterized by one of the physical properties [in]selected from the group consisting of size, density, color and shape.

3. (once amended) The method [as claimed in]of claim 2, wherein each particle sub-class is characterized by one of the physical properties [in the group]selected from the group consisting of size, density, color and shape, but is difference from the property characterizing the particle class.

4. (once amended) The method [as claimed in]of claim 1, wherein the mixtures are formed by mixing two particle classes or sub-classes in a ratio such that the difference in [number of one class with respect to the other is detectable in the identifying step]the relative amount of each class or sub-class can be used to determine the particle class to which the target substance is bound.

5. (once amended) The method [as claimed in any preceding claim]of claim 1, wherein the target substance is marked so as to be detectable.

6. (once amended) The method [as claimed in]of claim 5, wherein the [marking is performed]target substance is marked by attaching a moiety selected from the group consisting of a fluorescent moiety, a radioactive moiety, a colored moiety.

7. (once amended) The method [as claimed in]of claim 5, wherein the target substance reacts with the ligand to which it binds to provide a detectable effect, such as fluorescence or color.

8. (once amended) The method [as claimed in any preceding claim]of claim 1, wherein the identification is performed by ocular inspection under microscope.

9. (once amended) The method [as claimed any preceding claim]of claim 1, wherein the identification is performed by image analysis with a computer.

10. (once amended) The method [as claimed in any preceding claim]of claim 1, wherein said mixture is exposed to at least two target substances.

11. (once amended) A library of different ligands, comprising particles belonging to a plurality of particle classes, each particle class being distinguishable from [another class]the other particle classes by at least one physically distinguishable property, each particle class being comprised of at least two [subclasses]sub-classes, wherein each [subclass]sub-class is distinguishable from [another subclass]the other sub-classes by [another]a physical property of the particle class

from the property which distinguishes the particle class from other classes within the set, and further wherein[, different from the property(ies) of said particle classes,] those particles belonging to [one and]the same sub-class having at lease one type of ligand attached to their surface.

12. (once amended) The ligand library [as claimed in]of claim 11, wherein one of said properties of the particle classes or sub-classes is the size[, suitably the diameter] of the particles.

13. (once amended) The ligand library [as claimed in]of claim 11, wherein one of said properties of the particle classes or sub-classes is the density of the particle.

14. (once amended) The ligand library [as claimed in]of claim 11, wherein one of said properties of the particle classes or sub-classes is the shape of the particle.

15. (once amended) The ligand library [as claimed in]of claim 11, wherein one of said properties of the particle classes or sub-classes is the color of the particle.

Claims (clean version encompassing amendments)

1. (once amended) A method of identifying one or more substances having affinity for a given target substance, comprising:

(i) providing a set of particle classes, each said particle class being distinguishable from other classes within the set by at least one physical property, each particle class being comprised of at least two sub-classes, wherein each sub-class is distinguishable from the other sub-classes of said particle class by a physical property, which is different from the property which distinguishes the particle class from other classes within the set, and further wherein each sub-class contains particles having at least one of said one or more substances attached to the surface thereof as a ligand, said ligands being different from ligands attached to particles of other particle classes or sub-classes;

(ii) combining a plurality of classes or sub-classes to form at least one mixture,

(iii) distributing said at least one mixture in separate vessels;

(iv) exposing said mixture in said separate vessels to said target substance;

(v) removing all target substance not having bound to any ligand; and

(iv) identifying to which particle classes or sub-classes said target substance actually has (have) bound, by

identifying in which vessel or vessels target substance has bound to particles in the mixture present in said vessel or vessels, identifying to which particle class said target substance has bound; and

identifying to which particle class or sub-class said target substance has bound.

2. (once amended) The method of claim 1, wherein each particle class is characterized by one of the physical properties selected from the group consisting of size, density, color and shape.

3. (once amended) The method of claim 2, wherein each particle sub-class is characterized by one of the physical properties selected from the group consisting of size, density, color and shape, but is difference from the property characterizing the particle class.

4. (once amended) The method of claim 1, wherein the mixtures are formed by mixing two particle classes or sub-classes in a ratio such that the difference in the relative amount of each class or sub-class can be used to determine the particle class to which the target substance is bound.

5. (once amended) The method of claim 1, wherein the target substance is marked so as to be detectable.

6. (once amended) The method of claim 5, wherein the target substance is marked by attaching a moiety selected from the group consisting of a fluorescent moiety, a radioactive moiety, a colored moiety.

7. (once amended) The method of claim 5, wherein the target substance reacts with the ligand to which it binds to provide a detectable effect, such as fluorescence or color.

8. (once amended) The method of claim 1, wherein the identification is performed by ocular inspection under microscope.

9. (once amended) The method of claim 1, wherein the identification is performed by image analysis with a computer.

10. (once amended) The method of claim 1, wherein said mixture is exposed to at least two target substances.

11. (once amended) A library of different ligands, comprising particles belonging to a plurality of particle classes, each particle class being distinguishable from the other particle classes by at least one physically distinguishable property, each particle class being comprised of at least two sub-classes, wherein each sub-class is distinguishable from the other sub-classes by a physical property of the particle class from the property which distinguishes the particle class from other classes within the set, and further wherein those particles belonging to the same sub-class having at lease one type of ligand attached to their surface.

12. (once amended) The ligand library of claim 11, wherein one of said properties of the particle classes or sub-classes is the size of the particles.

13. (once amended) The ligand library of claim 11, wherein one of said properties of the particle classes or sub-classes is the density of the particle.

14. (once amended) The ligand library of claim 11, wherein one of said properties of the particle classes or sub-classes is the shape of the particle.

15. (once amended) The ligand library of claim 11, wherein one of said properties of the particle classes or sub-classes is the color of the particle.

16. The use of a ligand library as claimed in claim 11 for screening purposes.

COMBINATORIAL LIBRARY WITH PARTICLE CLASSES THAT CAN BE DISTINGUISHED BY TWO FEATURES E.G. SIZE, DENSITY, COLOR, ETC.

The present invention relates generally to screening methods and selective identification of target substances.

Background of the invention

In the pharmaceutical area i.a. it is often necessary to test very large numbers of substances against one or more target compounds to find out if any of the tested substances possess affinity towards the target, or in some other way interacts with the target. This normally is performed by binding each candidate compound (as a ligand) to some matrix e.g. a particle, loading a number of the particles in one reaction vessel of some kind, and incubating with the target substance. Either the candidate compound (ligand) or the target is marked with identifiable groups such as fluorescent or radioactive groups or nuclei. After the reaction is deemed to have come to completion analysis of the vessel is performed to see if the marker substance or group is present, indicating that the target has indeed reacted with the substance bound to the matrix.

Screening large numbers of substances of course requires large numbers of vessels, which is laborious and tedious to handle. 10000 compounds would require 10000 individual reaction vessels to be handled.

Another possibility is to mark particles with say 10000 different ligands. A mixture is prepared comprising particles having 100 different ligands and the mixture is placed in a well of a microtiter plate. 100 such mixtures are prepared totaling 10000 different ligands and said mixtures are placed in one well each. All wells are exposed to and incubated with the target substance marked with a fluorescent moiety. If fluorescence is detected in one well one knows that a hit is present in that well. A second experiment is performed where the 100 different ligands in said well are individually placed in one well each, and the incubation and exposure is repeated. The well exhibiting fluorescence will then contain the desired substance. Thus, it is necessary to perform two sets of experiments.

Therefore, it would be desirable to have access to methods and means for rendering such screening procedures less time and space consuming, and to enable the procedure to be carried out in one step.

US-5,858,670 relates to identification by sequencing of peptides bound to beads. The method requires many operations. It mentions staining of beads (col. 41, lines 47-54) for identification. This particular aspect per se, does not form a part of the present invention

In "One-Bead-One-Structure Combinatorial Libraries", Biopolymers, Vol. 37, 177-198, there is disclosed a very complicated (many steps required) method of making libraries for the identification of binding to specific ligands. The actual screening procedure requires a large number of iterations.

US-5,817,751 discloses libraries where beads have "identifier tags". These tags can be "microscopically or otherwise distinguishable features", (size, shape, mass, charge, color). See col. 23, lines 10-21, and lines 42-46. The screening process involves releasing the tags from the beads and subsequent amplification thereof.

EP 0 773 227 A1 discloses "identifier tags", meaning some "physical attribute" (examples given are size, shape, color, optical density) by which a solid support (e.g. a bead) can be identified and distinguished. See p 5, lines 17-33. The screening procedure is conventional, and involves sorting out fluorescing beads by using a cell sorting instrument (FACS).

US-5,162,863 discloses the possibility of using particles of different sizes for identification purposes.

Summary of the Invention

The present invention seeks to provide a method of identifying one or more substances having affinity for a given target substance, that would require a smaller number of reaction vessels than what is necessary today, and also a smaller number of operations, thereby speeding up the process of investigation substantially.

This object is achieved by the method according to the invention, whereby a combination of distinguishable properties of particles to which ligands are attached, is used for identification of those particles to which a target substance has bound. The method is defined in claim 1.

By providing a class of particles having one property in common, and subdividing each such class into sub-classes having another property, it becomes possible to screen a very large number in only two steps.

In a second aspect of the invention there is provided a library of ligands, comprising individually distinguishable particles of a matrix material, having ligands attached to the surface, and being suitable for binding candidate compounds (ligands), and usable in screening procedures. This aspect is defined in claim 11.

A third aspect of the invention is the use in screening procedures, of a ligand library comprising individually distinguishable particles of a matrix material, suitable for binding candidate compounds, which is characterized by using fact that the particles are distinguishable, as a marker for each said candidate compound. The library is defined in claim 16.

Furthermore, in preferred embodiments, selecting the distinguishable property to be different particle size in combination with density, is very powerful in this respect.

Brief Description of the Drawings

The invention will now be described with reference to examples and to the drawings, in which

Fig. 1 is a schematic representation of a micro titer well in which a mixture of 100 different particle-ligand combinations has been incubated;

Fig. 2 is another schematic representation of a micro titer well;

Fig. 3 is photograph of the contents of a microtiter well taken through a fluorescence microscope; and

Fig. 4 is a graphical output from an image analysis.

Detailed Description of Preferred Embodiments

For the purpose of this application, the term "particle class" is taken to mean particles having at least one property distinguishing them from other particles of another "class". One such property can be size (diameter), another the density. Still another kind of "particle class" is formed when a number of particles of one class having a first ligand attached, is used together with particles of the same class but having a second ligand attached thereto, but where the ratio of the number of particles having different ligands is different, e.g. 2:1.

A further particle class is formed when a coordinate is assigned to a set of particles

The term "sub-class" is taken to mean particles having as common features, the features or properties of a "particle class", and having been treated in some way so as to be distinguishable from another "sub-class". A "sub-class" is e.g. formed when particles of one size are provided with one ligand, and another "sub-class" is formed when the same particles are provided with a second ligand, different from the first.

Within one and the same class, i.e. particles having the same size, "sub-classes" are also formed by providing particles with e.g. different density but having the same diameter.

The term "ligand" shall mean one moiety attached to a particle, or a group of such moieties.

The basic idea behind the invention is the insight that in a mixture of objects, wherein groups of objects in said mixture have some distinguishable and identifiable feature in common within each group, it is easy to identify each group of objects. Also it is easy to identify if there has been a change in the property of the objects in one group. If for example a mixture consists of all white golf balls, tennis balls and footballs, it is very easy by visual inspection to determine that say the golf balls have changed color to green.

This insight has been applied by the inventor in the field of screening for substances having some desired property amongst large numbers of candidate substances, or ligands.

An extremely simplified example is the case where two ligands are to be examined for affinity to a target substance suitably marked, e.g. with a fluorescent marker. Each ligand would then be bound to particles with mutually different size and then the particles are mixed. The mixture is placed in well of a micro titer plate and incubated. After incubation the well is washed. If the target selectively binds to one ligand it will be an easy matter to identify which ligand has bound since one can measure the size of the fluorescing particles and thereby determine which the ligand is.

As another simplified and illustrative but slightly more complex example, let us assume that one is interested in testing four ligands for affinity to some macro-molecule, e.g. a protein. Let us also assume that each different ligand is individually bound to a particle. Furthermore we assume that the particles are of only two different classes, in this case we assume two different particle sizes. Two mixtures are prepared, each comprising two sets of particles having distinguishable size, and having different ligands bound thereto. The two mixtures are placed in one well each and incubated with the macro-molecule, appropriately marked with e.g. a fluorescent marker. Thus, we have two wells each containing the same two different particle classes, but having mutually different ligands bound to them. After incubation the wells are washed to remove non-bound substances. If the macro-molecule binds to one ligand it will be a straight forward matter to identify which one by determining which particle size and which well exhibits fluorescence. This is most easily done under a fluorescence microscope, and can be done by ocular inspection in a case like the described, where there are only few wells and few ligands present. Other methods of detection are available, e.g. so called flow cytometry in combination with Coulter-counter techniques. These methods are well known to the skilled man and need not be discussed in detail herein.

For production screening using large numbers of ligands and particles in large numbers of wells, image analysis by computer using specialized software is more feasible. An example of such commercial software is LEICA® Q 500 MC.

The skilled man will appreciate that the principle demonstrated above is applicable to larger numbers of different particle sizes and large numbers of wells, as will be explained by further examples below.

The basic principle of a first embodiment of the invention may be said to encompass identification of a ligand having reacted with a target substance, by two parameters, namely a) particle size, and b) coordinate for the particle on the micro titer plate (simply in which well it is present).

Still another property of the particles that is usable as an identifying "marker" is the density. It is relatively easy to make particles having well defined densities. If ligands are bound to such particles, mixtures of said particles can be separated by centrifugation in a density gradient, and fluorescent bands in the test tube after centrifugation can be easily determined, and the position along the tube will indicate which density fraction is a hit.

The basic principle can be further elaborated by making mixtures containing different number ratios between particles of the same size having different ligands, and providing them in the same well. Thus, if two different ligands are bound to one and the same particle size but in two fractions, and these two fractions are mixed e.g. in a ratio 1:2, of course it will be an easy matter to determine to which fraction the target has bound by the fluorescence intensity being either 1/3 or 2/3 of the maximum possible, if all particles would have fluoresced.

The particles to be used may be made of any material to which suitable ligands of interest can be attached or bound. The particles are preferably hydrophilic and built up of one or more polymers which are insoluble in water. Hydrophobic polymers that have been derivatized to become hydrophilic are included. Suitable polymers are polyhydroxy polymers, e.g. based on polysaccharides, such as agarose, dextran, cellulose, starch, pullulan, etc. and completely synthetic polymers, such as polyacrylic amide, polymethacrylic amide, poly(hydroxyalkylvinyl ethers), poly(hydroxyalkylacrylates) and polymethacrylates (e.g. polyglycidylmethacrylate), polyvinylalcohols and polymers based on styrenes and divinylbenzenes, and copolymers in which two or more of the monomers corresponding to the above-mentioned polymers are included. Polymers, which are soluble in water, may be derivatized to become insoluble, e.g. by cross-linking and by coupling to an insoluble body

via adsorption or covalent binding. Hydrophilic groups can be introduced on hydrophobic polymers (e.g. on copolymers of monovinyl and divinylbenzene) by polymerization of monomers exhibiting groups which can be converted to OH, or by hydrophilization of the final polymer, e.g. by adsorption of suitable compounds, such as hydrophilic polymers.

The particles can also be based on inorganic material, such as silica. Preferred particles lack hydrolytically unstable groups, such as silane, ester and amide groups.

The particles may be porous.

The term "hydrophilic particle" in practice means that the accessible surface of the particles is hydrophilic in the sense that can be penetrated by aqueous liquids. Typically the accessible surfaces on a hydrophilic particle expose a plurality of polar groups for instance comprising oxygen and/or nitrogen atoms. Examples of such polar groups are hydroxy, amino, carboxy, ester, ether of lower alkyls (such as $(-CH_2CH_2O-)_nH$ where n is an integer).

Suitable size fractions of such particles are obtained by sieving, using standard methods well known to the skilled man. The resulting particle fractions will have some spread in terms of average diameter, but the spread can be controlled so that overlap between fractions can be adequately controlled.

Furthermore, a certain overlap in fraction size is no problem since the image analysis software that calculates average particle sizes is able to distinguish with a high degree of certainty to which fraction the fluorescing particles belong.

Another possible material for the particles is poly-styrene. By using this material it is possible to make mono-disperse particles without overlap in fraction size.

In order to prepare suitable mixtures when the number of particles and ligands is large, so called factor design is preferably used. The theory behind this methodology is disclosed in *"Statistics for Experiments"* by Box et al, ISBN 0-471-09315-7.

The invention will now be further described by way of the following non-limiting examples.

EXAMPLES

Example 1

10 particle fractions ranging in size from fraction 1 of 10-20 μm up to fraction 10 of 100-110 μm are prepared. Each size fraction is further subdivided into 10 density fractions ranging from 1,01 up to 1,19 with increments of 0,02. Thus, in all 100 different sub classes of particles defined by both a) size and b) density is produced. To particles of each sub class a different ligand is bound to provide 100 unique combinations of ligand and particle.

10000 different ligands are to be tested. Thus, the 100 particle subclasses are used to provide 100 sets of 100 particle-ligand combinations. Each set of 100 combinations is placed in one well each of a 100-well microtiter plate. Then all wells are incubated with a target compound. The compound is suitably marked with a fluorescent moiety. In addition to being identifiable by the two properties of the particle (size and density), each ligand is identified by a coordinate, i.e. the well number.

After incubation the wells are washed to remove all target compounds that have not bound to any ligands.

Fig. 1 shows a matrix symbolizing one well in which it has been determined that the size fraction 70-80 μm exhibits fluorescence (symbolized by the particles being filled), and thus contains a subclass of particles to which the target compound has bound. Of course only 10% of the particles of this size will fluoresce, but it cannot be decide which ligand has reacted.

In order to determine which density fraction contains the target bound to the particle, the entire contents in the well is centrifuged in a density gradient, so as to yield bands corresponding to each density. The band (density 1,11) containing the fraction having target bound thereto will then fluoresce. This is symbolized by the dots representing the density fraction in question being filled.

The cross section of the two rows will identify the particular ligand that has bound to the target molecule.

Example 2

In order to double the number of possible ligands to test (or alternatively to reduce the number of wells needed for the screening), it is also possible to use the number ratio between subclasses of particles as a marker.

Thus, 10 particle fractions ranging in size from fraction 1 of 10-20 μm up to fraction 10 of 100-110 μm are prepared. Each size fraction is further subdivided into 10 density fractions ranging from 1,01 up to 1,19 with increments of 0,02. Thus, in all 100 different sub classes of particles defined by both a) size and b) density is produced. To particles of each sub class a different ligand is bound to provide 100 unique combinations of ligand and particle.

20000 different ligands are to be tested. To this end, the 100 particle subclasses are used to provide a first lot of 100 sets of 100 particle-ligand combinations, which yields 10000 combinations. Furthermore, a second lot of 100 sets of 100 particle-ligand combinations, but with 10000 other ligands than in the first lot are made. In one and the same well one set of 100 particle-ligand combinations from the first lot is combined with twice as much (total number of particles or weight of the mixture or some other measure of quantity) from the second lot. This means that for each individual particle (having a unique size and density), there will be two possible ligands. The well is then incubated with a target molecule suitably marked.

After washing the wells to remove the non-bound target molecules, it is found that the size fraction 30-40 μm exhibits fluorescence (see Fig 2 which is a schematic representation of the contents of the well). It is also determined by simple counting or by measuring a total intensity of the fluorescence, that the number of fluorescing particles is twice as many as would have been the case if the target had reacted with particles from the first lot, and thus it must be from the second lot.

A separation by density through centrifugation is performed as in Example 1, yielding 10 bands, one of which is fluorescing (density 1,01). Again, all information needed to determine which ligand has reacted is available.

Example 3

In this example it is demonstrated that the number of possible ligands to test can be increased also by mixing the particle classes in an intelligent way.

Particles of three different particle sizes, average diameters being 15 μm, 25 μm and 55 μm respectively are used and 18 different ligands (designated A-S) are bound to these particles, thus forming 54 different combinations of ligand/particle. Thus, a combination of ligand A with a particle having the diameter 15 μm is designated A-15, ligand B bound to a 55 μm particle is designated B-55 etc.

Mixtures of these ligand-particle aggregates are prepared according to the following protocol:

Mixture No.	Composition (ligand/particle size)
1	A-15; B-25; C-55; D-15; E-25; F-55
2	G-25; H-55; I-15; K-25; L-55; M-15
3	N-55; O-15; P-25; Q-55; R-15; S-25
4	A-15; B-25; C-55; D-25; E-55; F-15
5	G-25; H-55; I-15; K-55; L-15; M-25
6	N-55; O-15; P-25; Q-15; R-25; S-55

According to this protocol, a combination can occur either in one well only, or in two different wells. For example the combination A-15 occurs in well No. 1 and 4, whereas combination D-15 occurs only in well No. 1.

These mixtures are placed in one well each (designated with the same number as the mixture numbers) of a micro titer plate, thus in six wells. The mixtures are incubated with one target substance, suitably marked with a fluorescent moiety. After completed incubation the wells are washed with water to remove all unbound target substances.

It is found that wells No. 2 and 5 exhibited fluorescence, and that the fluorescent particles have a diameter of 25 μm by image analysis. From this information it can be concluded that ligand G has bound to the target molecule, since the only combination with a particle of diameter 25 μm common to well 2 and 5 is G-25. This being an extremely simple example, it is appreciated that the protocol of mixing can be much more sophisticated and involved, in order to distinguish one reacting species among a large number of possibilities.

In Fig. 3 a typical image viewed in a fluorescence microscope is shown. As can be clearly seen it is possible even with ocular inspection to distinguish three different particle sizes (in this case for illustrative purpose, all particles fluoresce in order to be able to see them; in a real run of course only one particle size should be visible). An image analysis by computer yields the result shown in Fig. 4, wherein also the distribution of sizes within each nominal class can be seen.

In a further development of the method, the mixtures of ligand-particle combinations are incubated with two or more target molecules. To this end the targets are suitably marked with distinguishable markers, such as fluorescent moieties exhibiting fluorescence of different wave lengths.

What is claimed is:

1. A method of identifying one or more substances having affinity for a given target substance, comprising:

providing a set of particle classes, each said particle class being distinguishable from another class by at least one physical property, each particle class being comprised of at least two subclasses, wherein each subclass is distinguishable from another subclass by another physical property, different from the property of said particle classes, each subclass comprising particles having at least one of said substances attached to the surface thereof as a ligand, said ligands being different from ligands attached to particles of other particle classes or subclasses;

combining a plurality of sub-classes to form at least one mixture,

distributing the mixtures in separate vessels;

exposing each mixture to said target substance;

washing away all target substance not having bound to any ligand; and

identifying to which particle sub-class(es) said target substance actually has(have) bound, by the following steps:

identifying in which vessel or vessels target substance has bound to particles

in the mixture present in said vessel or vessels, identifying to which particle class said target substance has bound; and

identifying to which particle sub-class said target substance has bound.

2. The method as claimed in claim 1, wherein each particle class is characterized by one of the properties in the group size, density, color and shape.

3. The method as claimed in claim 2, wherein each particle sub-class is characterized by one of the properties in the group size, density, color and shape, but is different from the property characterizing the particle class.

4. The method as claimed in claim 1, wherein the mixtures are formed by mixing two particle classes in a ratio such that the difference in number of one class with respect to the other is detectable in the identifying step.

5. The method as claimed in any preceding claim, wherein the target substance is marked so as to be detectable.

6. The method as claimed in claim 5, wherein the marking is performed by attaching a moiety selected from the group consisting of a fluorescent moiety, a radioactive moiety, a colored moiety.

7. The method as claimed in claim 5, wherein the target substance reacts with the ligand to which it binds to provide a detectable effect, such as fluorescence or color.

8. The method as claimed in any preceding claim, wherein the identification is performed by ocular inspection under microscope.

9. The method as claimed any preceding claim, wherein the identification is performed by image analysis with a computer.

10. The method as claimed in any preceding claim, wherein said mixture is exposed to at least two target substances.

11. A library of different ligands, comprising particles belonging to a plurality of classes, each particle class being distinguishable from another class by at least one physically distinguishable property, each particle class being comprised of at least two subclasses, wherein each subclass is distinguishable from another subclass by another physical property,

different from the property(ies) of said particle classes, those particles belonging to one and the same sub-class having at least one type of ligand attached to their surface.

12. The ligand library as claimed in claim 11, wherein one of said properties of the particle classes or sub-classes is the size, suitably the diameter of the particles.

13. The ligand library as claimed in claim 11, wherein one of said properties of the particle classes or sub-classes is the density of the particle.

14. The ligand library as claimed in claim 11, wherein one of said properties of the particle classes or sub-classes is the shape of the particle.

15. The ligand library as claimed in claim 11, wherein one of said properties of the particle classes or sub-classes is the color of the particle.

16. The use of a ligand library as claimed in claim 11 for screening purposes.

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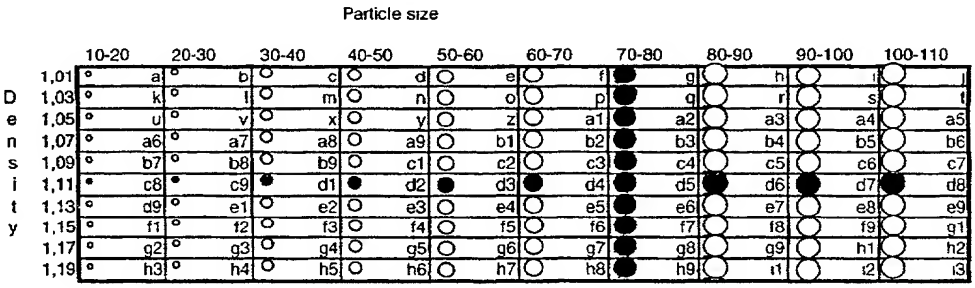
(54) Title: COMBINATORIAL LIBRARY WITH PARTICLE CLASSES THAT CAN BE DISTINGUISHED BY TWO FEATURES E.G. SIZE, DENSITY, COLOR, ETC.



(57) Abstract: The invention relates to a method of identifying one or more substances having affinity for a given target, comprising providing a set of particle classes, each said particle class being distinguishable from another class by at least one property. The particles belonging to one and the same class may have another property that distinguishes them, e.g. different densities, thus forming sub-classes. To each unique sub-class a number of unique ligands can be attached to sets of particles forming further sub-classes. A plurality of sub-classes is combined to form at least one mixture. The mixtures are distributed in separate vessels and exposed to said substance. All target substances not having bound to any candidate substance are washed away, and it is determined to which particle sub-class(es) said target substance actually has (have) bound.

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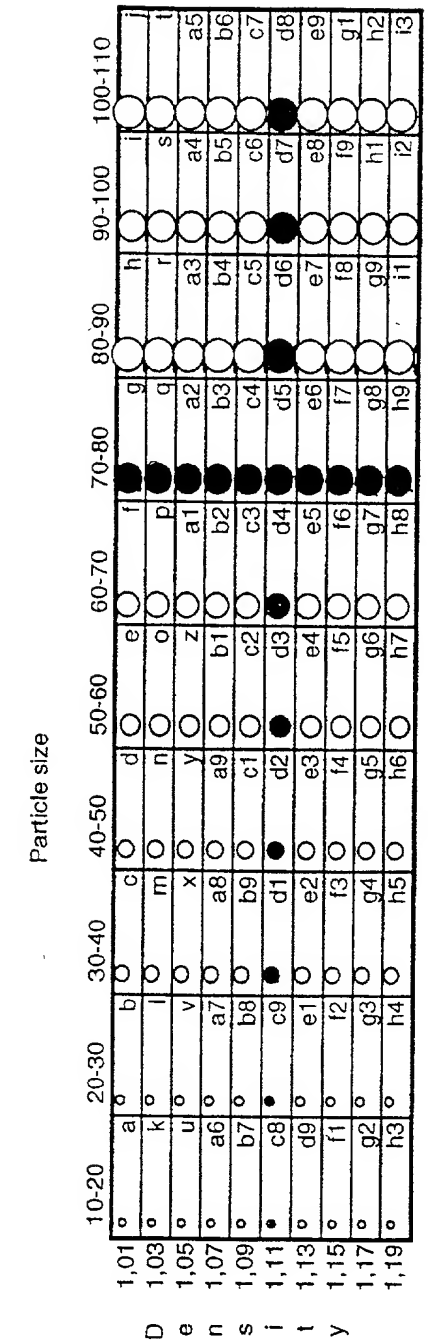
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30-40
40-50
50-60
60-70
70-80
80-90
90-100
100-110
Density
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1,11
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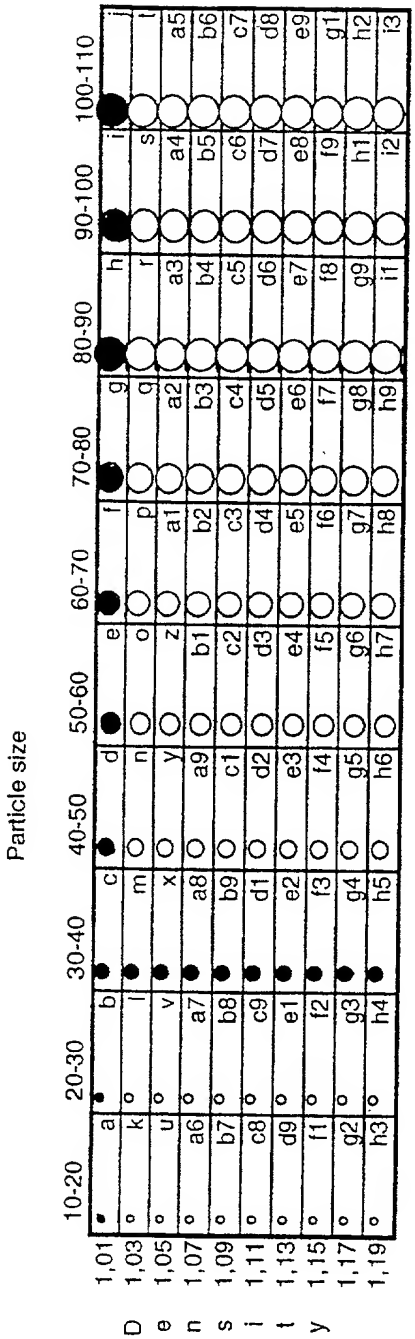
1,15
1,17
1,19

Fig. 1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90-100
100-110
Density
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1,11
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1,15
1,17
1,19

Fig. 2

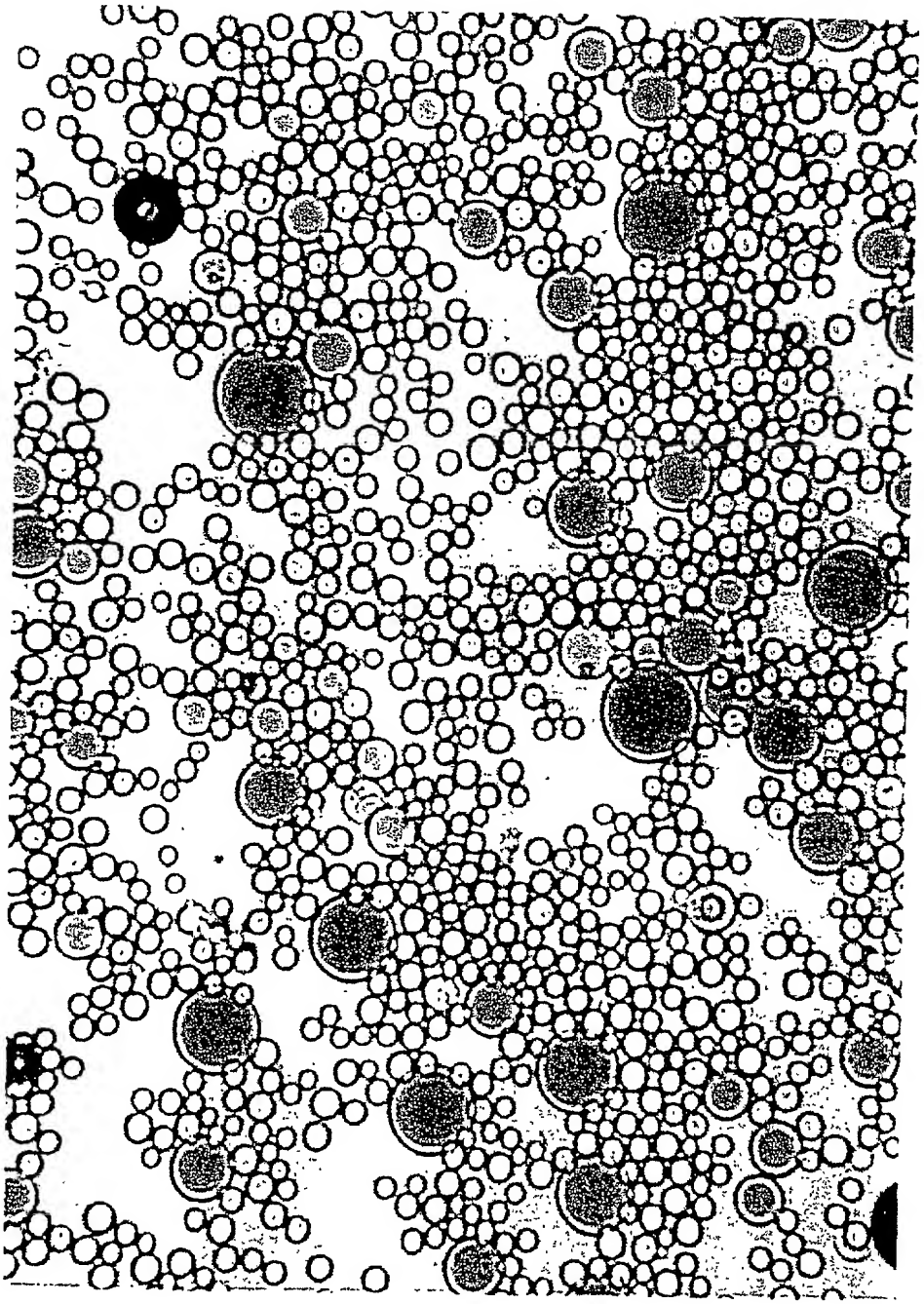

Fig. 3

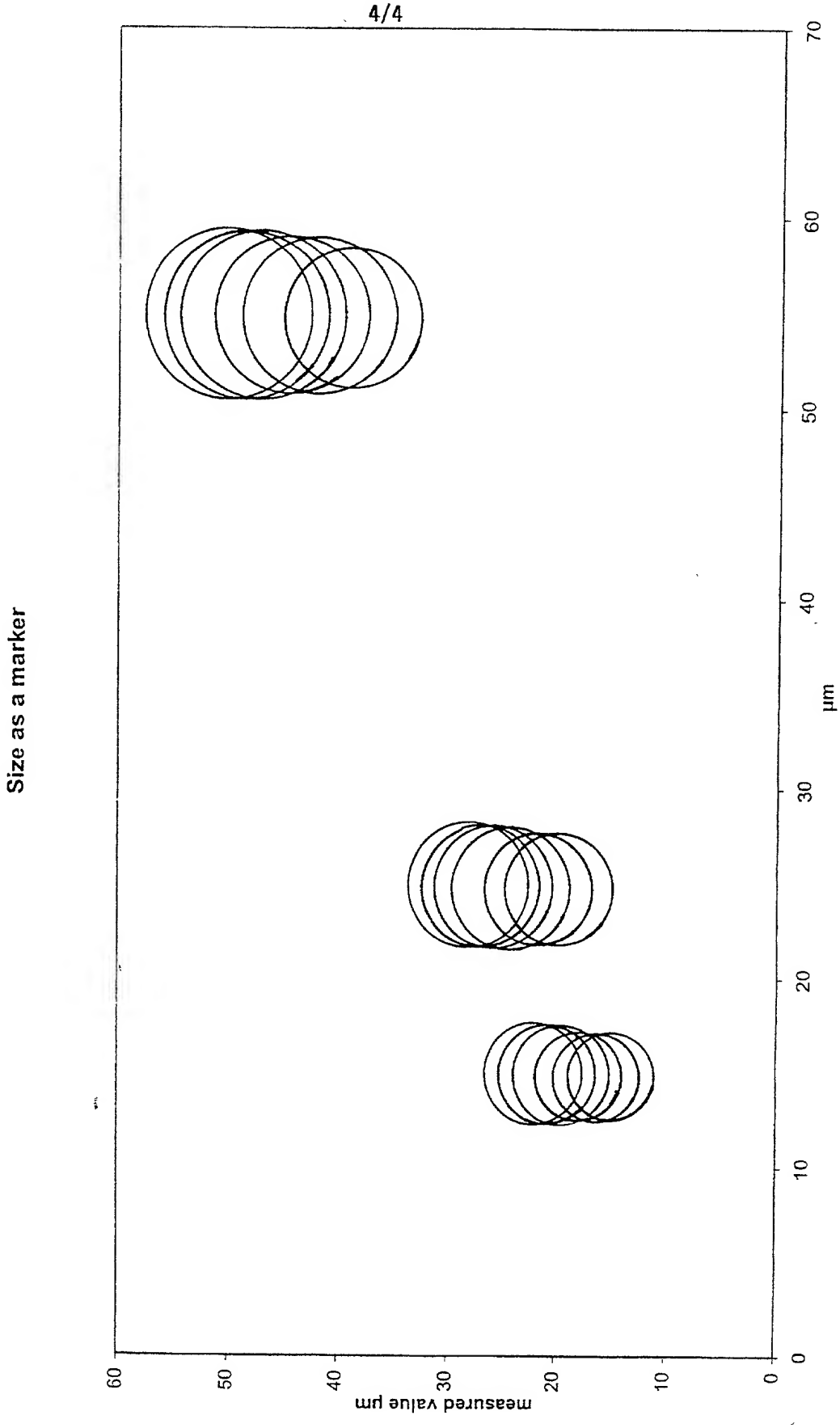
Size as a marker
measured value µm
0
10
20
30
40
50
60
µm
0
10
20
30
40
50
60
70

Fig. 4

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	PU-9925
First Named Inventor	Norrman
COMPLETE IF KNOWN	
Application Number	10 / 018,042
Filing Date	10-Dec-2001
Group Art Unit	To be assigned
Examiner Name	To be assigned

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

Combinatorial Library with Particle Classes that can be Distinguished by Two Features e.g. Size, Density, Color, etc.

(Title of the Invention)

the specification of which
☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 06/21/2000 as United States Application Number or PCT International Application Number PCT/SE00/01174 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
9902479-6	Sweden	06/30/1999	☐	☐	☒
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)

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I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*
PCT/SE00/01174	06/21/2000	

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Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
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City		State		ZIP		Country	

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1-00